Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

produced abortion in 26 days. Intravaginal application of the same material in another pregnant female guinea-pig was without result. Subcutaneous inoculation of a broth suspension of infected placenta (Case 3) into a pregnant female guinea-pig produced abortion in 10 days, and the subcutaneous inoculation of a pure culture of B. abortus isolated by the Nowak plate method from the same placenta caused a premature delivery after three and a half days in one guinea-pig and an abortion eight days after inoculation in a second guinea-pig. From this last animal the bacillus was recovered from the point of inoculation, from the interior of the uterus, and from each of the two placentae.

From this rather limited series of examinations and experiments we may conclude that the bacillus of Bang is the microbic cause of at least some of the contagious abortion of cattle in this country. We hope this report may prove of some value to workers in this particular field, and that it may also serve to draw the attention of others to the very interesting biological properties of this peculiar microorganism.

SOME OBSERVATIONS ON THE WASSERMANN REACTION.*

LAWRENCE T. CLARK.

(From the Research Laboratories of Parke, Davis & Co., Detroit, Mich.)

AN esteemed early teacher once said: "The bacteriologist of yesterday was one who isolated and cultivated bacteria and attempted to classify them; the bacteriologist of tomorrow must be one who cannot only grow these microorganisms, but measure the scope and products of their activities and offer means for correcting their evil effects." In the serum diagnosis of syphilis, wherein the phenomenon known as the fixation of complement is employed, the products of parasitic infection have been detected and measured before the bacteriologist has succeeded with the more fundamental procedure as the isolation and growth of the Treponema pallidum.

The value and importance of the complement fixation reaction to the clinician and, indirectly, to the public, and difficulties attending its extensive use have been repeatedly set forth. I believe these facts are sufficiently familiar to all to need no further comment at this time. Modifications of the original Wassermann method, substitutions for some of the various ingredients of this test, and reactions depending on different phenomena have been exploited by enthusiastic workers who have striven to perfect a test which would be within reach of the average clinician. In the main the use of these so-called modifications leaves the doubtful case still doubtful and gives definite results only where such a test is not needed. The competent practitioner requires aid from a reliable test in his doubtful cases only; those with a known history and those with clearly diagnostic symptoms need no verification. The more urgent need, then, is for a test upon which we can place absolute dependence in the doubtful case in which accurate diagnosis would mean much to the physician and more to the patient, to whom an error in diagnosis might bring lasting injury.

The questions then arise, how and by whom shall the serum test for syphilis be made? Can the practicing physician with a few cases

---

* Received for publication March 7, 1910.

each month carry out the original test or some reliable modification and do justice to his doubtful case?

Among the more commendable modifications employed by various workers, that of Noguchi[1] renders the test less complicated and, according to Fox,[2] more accurate than the original Wassermann. The necessary reagents, reduced to a more or less stable condition, are thus made available for making the test at the bedside if necessary.

During the past two years the author has tested 494 bloods by different methods and has arrived at a test, slightly modified from the original Wassermann, upon which he places considerable reliance. The first 392 samples were tested with precipitin reagents in various combinations for the precipitin reaction. It was hoped that a method simpler than that depending upon complement fixation could be worked out, which would give a reaction easy to read and reliable when carried out by the practicing physician. Results obtained with the 392 cases, the majority of which were diagnosed clinically, gave a percentage of less than 70 per cent of reliable findings. While the clinician having access to a large number of cases may be able to detect slight differences between questionable and normal bloods, the precipitin tests are too delicate for him who most needs such and has but few cases at his command.

The color test described by Schurmann[3] was also tried, but gave very unsatisfactory results. I was unable to confirm the findings of the writer.

Owing to lack of success with the methods tested and in order to control results obtained with precipitin reagents, the original Wassermann reaction was taken up and in a somewhat modified form has been used since. The five factors as employed are:

1. Patient's serum inactivated by heating at 56° C., ½ hr.
2. Antigen—alcoholic extrac of syphilitic liver.
3. Complement—fresh guinea-pig serum.
4. Hemolytic antigen—washed red corpuscles from sheep, diluted 1:100 in salt solution.
5. Hemolytic amboceptor—anti-sheep-corpuscle rabbit serum.

One standard drop of patient's serum, known normal serum, and known syphilitic serum were put into three separate tubes (5 cm. × 0.8 cm.). To each of the three tubes one unit of antigen was then added and the tubes shaken; 0.02 c.c. of fresh normal guinea-pig serum was next added, the mixture shaken thoroughly and incubated 20 minutes. Immediately upon removing the tubes from the incubator 1 c.c. of sheep washed red corpuscles (1:100) and 1 unit (1/625 c.c.) inactive hemolytic serum were placed in the tubes. They were again shaken and allowed to stand 10 minutes at room temperature when readings were taken.

---

[1] *Jour. Exper. Med.*, 1909, 11, p. 392.

[2] *Jour. Cutan. Dis.*, 1909, 27, p. 338.

[3] *Deut. Med. Wchnschr.*, 1909, 35, p. 616.

Several sets of tests, carried out according to this method with the complement from the same guinea-pig, gave entirely concordant results. Complete hemolysis took place in the normal controls within 10 minutes, while no hemolysis occurred in the syphilitic tubes. Believing at that time that the test as outlined was adapted to the purpose intended, I planned to run a number of samples independently to test the accuracy and uniformity of the method before comparing results with those obtained with precipitin reagents.

Samples of the serum bearing numerals or letters only were very kindly furnished me by Dr. H. R. Varney and Dr. Andrew D. Potter. Clinical data pertaining to cases were purposely withheld, consequently the laboratory tests were in no way influenced by them.

Difficulties were, however, encountered in changing the source of guinea-pig serum. The entire set of tubes frequently gave complete hemolysis, due to an excess of complement, or no hemolysis because of too little complement. In such instances the amount of complement was varied as the first results indicated, a retest made, and more reliable results were in that way obtained. Such a procedure is objectionable, as it exhausts the sample and takes more time than is necessary. My findings, as well as those of other workers, clearly indicating that fresh sera from different guinea-pigs possess decidedly variable amounts of complement, I at once undertook to correct this troublesome feature of the test and remove the necessity for making a retest.

A quantity of serum was obtained from a known active syphilitic whose serum had given good reactions and also from a normal individual whose serum had given no trace of a reaction by all methods employed. Sera were then obtained from a number of normal guinea-pigs of varying ages, and amounts varying from 0.02 to 0.04 c.c. taken to make the regular test as previously outlined. From these series of tests the amount of fresh guinea-pig serum necessary to cause complete hemolysis in 10 minutes with a normal serum was found to vary materially between 0.02 and 0.04 c.c.

From the above results this variation in that quality of guinea-pig serum subject to fixation accounts for the irregular results obtained when a constant amount of guinea-pig serum was used in earlier trials.

The limits, then, of the amount of complement to use in making a reliable test and the variation met with in the serum from different pigs call for a careful standardization of each lot of guinea-pig serum before using. While this procedure makes the test more complicated, the author has full confidence in the greater accuracy it gives.

To standardize the fresh guinea-pig serum, two series of three tubes each are taken; to one series one standard drop of inactive syphilitic serum is added per tube, and to the second series one standard drop of inactive normal serum is added per tube. One unit of antigen is then placed in each of the six tubes and shaken and 0.02, 0.03, and 0.04 c.c. of the guinea-pig serum is added to tubes 1, 2, and 3 of each series respectively, and all are then incubated 20 minutes. After incubation the hemolytic couple is added to all tubes and hemolysis watched for during the succeeding 10 minutes. The amount of guinea-pig serum necessary to give complete hemolysis in the normal tube with none in the corresponding syphilitic tube is then taken as the unit of complement in that particular guinea-pig serum and the regular test then carried out with the unknown samples. A convenient form of tube for measuring the guinea-pig serum was found in capillary pipettes, which were carefully graduated in hundredths by means of mercury.

One hundred and five sera were tested by the author's improved method just described. Of these samples 60 were from clinically diagnosed cases and 45 were doubtful; 57, or 95 per cent, of the known cases gave results corresponding to the clinical evidence; 42, or 93⅓ per cent, of 45 cases of doubtful diagnosis gave results substantiated by subsequent clinical findings; 8 cases with a positive initial reaction gave succeeding negative reactions after vigorous specific treatment.

Concerning the keeping qualities of the several factors used in making the test, I find that the hemolytic serum kept constantly at 1.6° C. slowly loses its strength. A lot made nine months ago is at present in use. Its titre when first taken was 0.002, which changed